

**IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE**

AFFYMETRIX, INC., a Delaware corporation,

Plaintiff/Counter-Defendant,

v.

ILLUMINA, INC., a Delaware corporation,

Defendant/Counter-Plaintiff.

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) Civil Action No.: 04-901 JJF
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PUBLIC VERSION

**ILLUMINA'S RESPONSE TO AFFYMETRIX'S STATEMENT OF DISPUTED MATERIAL FACTS
REGARDING ILLUMINA'S MOTION FOR SUMMARY JUDGMENT OF INVALIDITY OF THE
ASSERTED CLAIMS OF THE '432 PATENT**

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INTRODUCTION

Affymetrix's Statement misapplies the law and then cites to irrelevant facts in an attempt to cloud the conclusion that the SBH Abstract discloses each and every one of the limitations of the asserted claims of the '432 patent. But the key facts — facts that Affymetrix does not and cannot dispute — dictate that summary judgment of anticipation of the '432 patent is warranted. These undisputed facts include:

- The SBH Abstract discloses a plurality of beads;
- The SBH Abstract discloses beads with oligonucleotides attached to them;
- The SBH Abstract discloses beads with different oligonucleotides of the length and in the number set forth in the '432 patent claims;
- The SBH Abstract discloses beads being coded with an encoding system;
- The SBH Abstract discloses *the exact same encoding systems* as are mentioned in the '432 patent;
- Affymetrix's own witnesses have admitted time and again that all of the technology to make beads with oligonucleotides and encoding systems was known by 1989; and
- Five witnesses, including a member of Affymetrix's Scientific Advisory Board, confirm that the SBH Abstract was distributed widely and publicly at two conferences in the fall of 1989. There is not a single piece of evidence to the contrary.

Arguments about issues that have nothing to do with the asserted claims of the '432 patent, or attacks that the authors of the SBH Abstract were "crazy," do not create genuine issues of material fact. Applying the proper law to the facts listed above, there can be no doubt that (1) the SBH Abstract discloses every limitation of the asserted claims; (2) the SBH Abstract enables one of skill in the art to practice the '432 claimed invention; and (3) the SBH Abstract is

a printed publication under § 102(b). Summary judgment of anticipation is the only available conclusion.

I. AFFYMETRIX FAILS TO DISPUTE THAT THE SBH ABSTRACT DISCLOSES EVERY LIMITATION OF THE ASSERTED CLAIMS OF THE '432 PATENT

1. Affymetrix does not even attempt to dispute the following facts, as set forth in Illumina's Opening Brief and repeated below, which establish that the plain language of the SBH Abstract discloses every limitation of the asserted claims:

- **Claim 1:** The SBH Abstract discloses "a collection of beads comprising a plurality of beads which have binding polymers of different target specific sequence [*i.e.*, known DNA sequences] attached thereto." The SBH Abstract further discloses "beads being encoded with an encoding system," such as size, shape or color, or attaching to it a specific combination of known oligonucleotides, to identify the attached sequence by its association with the encoded bead. (Illumina Br. at 12-13)
- **Claim 2:** The SBH Abstract discloses a collection of beads with "oligonucleotides of [the same length]" by the example of all 12-mers, or oligonucleotides of 12 nucleotides long, attached to 10 million beads. (Illumina Br. at 14-15)
- **Claims 5 and 8:** The SBH Abstract discloses a collection of beads with "oligonucleotide sequences having the same number of nucleotides are at least 5 [or 10] nucleotides long" by using the example of 12-15mers, or oligonucleotides of 12 to 15 nucleotides long. (Illumina Br. at 15)
- **Claim 9:** The SBH Abstract discloses a collection of beads with "at least 10,000 of all the possible oligonucleotide sequences having the same number of nucleotides [] each attached to a different single bead" by its description of 10 million to 1 billion different beads each carrying a unique 12-15mer. (Illumina Br. at 16)

Testimony of five persons of skill in the art independently confirm that the SBH Abstract discloses every limitation of the asserted claims. (Illumina Br. at 6-7, 17-19)¹

2. While Affymetrix argues that "an encoding system" is not "adequately" disclosed, none of Affymetrix's "evidence" or arguments create a genuine dispute as to whether the SBH Abstract sufficiently describes this limitation under the law of anticipation. To the contrary, the SBH Abstract expressly describes the limitation "an encoding system whereby the target specific sequence of the polymer attached to the bead can be identified." ('432 claim 1) Specifically, the SBH Abstract describes how the beads are uniquely coded such that they can be distinguished from one another, and how the attached oligonucleotides can be determined as a result of their association with each coded bead. (Illumina Br. at 11, SBH Abstract Ins. 14-20) The SBH Abstract further describes — and Affymetrix admits — that beads can be coded by an encoding system based on "size, shape or color, or attaching to it a specific combination of known oligonucleotides." (*Id.* at Ins. 15-20; Affymetrix Stmt. ¶ 12) Such disclosure, regardless of whether it is in theory or practice, satisfies the "encoding system" limitation without dispute, and nothing further is required as a matter of law.

3. Affymetrix's arguments that the SBH Abstract was never implemented are irrelevant and depend upon a non-existent requirement that the SBH Abstract must have enabled its own invention. This is not the law of anticipation. It is well settled that "a claim is

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anticipated if each and every limitation is found either expressly or inherently in a single prior art reference." *Celeritas Tech., Ltd. v. Rockwell Int'l Corp.*, 150 F.3d 1354, 1361 (Fed. Cir. 1998) "[T]he law of anticipation does not require that the reference 'teach' what the subject matter of the patent teaches. . . . [I]t is only necessary that the claims under attack [] read on something disclosed in the reference." *Id.* at 1361 (citing *Kalman v. Kimberly-Clark Corp.*, 713 F.2d 760, 772 (Fed. Cir. 1983)). All of Affymetrix's arguments that the SBH Abstract does not disclose a "working system" are legally irrelevant and do not and cannot raise any genuine issue of material fact. (Affymetrix Stmt. ¶¶ 10-11) Indeed, these same arguments reappear in Affymetrix's enablement argument and are addressed below in section II.

4. Any further disclosure that Affymetrix argues is required — *e.g.*, arguments about lack of "any means of specific synthesis or attachment"² — are not limitations in the asserted claim limitations, and are thus irrelevant to the anticipatory disclosure of the SBH Abstract.

II. AFFYMETRIX'S ARGUMENTS THAT THE SBH ABSTRACT IS NOT ENABLING ARE LEGALLY MISGUIDED AND INCONSISTENT WITH ALL OF THE FACTS DEVELOPED DURING DISCOVERY

5. Affymetrix's arguments that the SBH Abstract is not enabled simply apply the wrong legal standard. First, Affymetrix argues that the SBH Abstract is not enabling because it was never implemented. (Affymetrix Stmt. ¶¶ 15-16, 21-23) As discussed above, this cannot create a genuine dispute as to any *material* fact because the law does not require this. In the case law that Affymetrix itself cites, the Federal Circuit has held that "[i]t is not [] necessary that an

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invention disclosed in a publication shall have actually been made in order to satisfy the enablement requirement." *Elan Pharms., Inc. v. Mayo Found. for Med. Educ. & Research*, 346 F.3d 1051, 1055 (Fed. Cir. 2003); *see also Bristol-Myers*, 246 F.3d at 1379 (citing *In re Donohue*, 766 F.2d 531, 533 (Fed. Cir. 1985)).³ Rather, "anticipation only requires that [the prior art disclosure of the claimed invention] be enabling to one of ordinary skill in the art." *Id.*

6. Ironically, while Affymetrix criticizes the SBH Abstract for not describing a working example, the '432 patent itself contains no working examples involving beads with oligonucleotides or encoding systems. (Illumina Br. Ex. A, '432 col. 67:38-69:12; 69:30-50)

7. Second, Affymetrix rehashes its erroneous argument that the SBH Abstract is not enabled because *its own invention* (i.e., the one in the SBH Abstract) as disclosed was not enabled:

Thus, the SBH Abstract itself, which purports to describe a theoretical method for sequencing the entire human genome, states that its goals would not be feasible given the state of the art as of its submission. By contrast, Illumina contends that [the SBH Abstract] would have enabled construction of *a device capable of sequencing the entire human genome* in 1989, or a few months thereafter.

(Affymetrix Stmt. ¶ 15 (emphasis added); *see also* ¶¶ 16-23) But again in Affymetrix's own cited authority, the Federal Circuit has made clear that "[t]o serve as an anticipating reference, the reference must enable *that which it is asserted to anticipate*." *Elan*, 346 F.3d at 1054 (emphasis added). In this case, therefore, the SBH Abstract must enable the *claims of the '432 patent* — "a collection of beads," which is as few as two beads for asserted claims 2, 5, and 8.

³ While an anticipatory reference must be enabled, "the standard for what constitutes proper enablement of a prior art reference for purposes of anticipation under § 102, however, differs from the enablement standard under § 112." *Rasmusson v. SmithKline Beecham Corp.*, 413 F.3d 1318, 1325 (Fed. Cir. 2005).

Whether the SBH Abstract invention was capable of accomplishing the more complex objectives of its authors — millions of beads to sequence the entire human genome in one experiment — is entirely irrelevant to whether the SBH Abstract enables and anticipates the claims of the '432 patent.

8. Affymetrix's desperation also shows when it specifically attempts to read non-existent limitations into the '432 patent claims to argue that the SBH Abstract is not enabled. Affymetrix argues there is no guidance regarding (1) visualization of hybridization events for a functional encoding system or (2) how the monolayer of beads would be attached to a surface in a manner that would allow multiple hybridizations. (Affymetrix Stmt. ¶¶ 18, 20) The asserted claims do not require hybridization or visualization of hybridization for an encoding system, and the '432 patent itself only discloses that "an encoding system may include a magnetic system, a shape encoding system, a color encoding system, or a combination of any of these..." (Illumina Br. Ex. A, '432 col. 21:61-64) Similarly, the asserted claims do not require a monolayer of beads, and the '432 patent actually contemplates encoded beads *in suspension*. (Illumina Br. Ex. A, '432 col. 21:52-61) Affymetrix's arguments are simply irrelevant to the enablement of the SBH Abstract as an anticipatory reference to the '432 patent, and do not raise any genuine issues of material fact.

9. The only point Affymetrix disputes as to enablement that has anything to do with the asserted claims is whether the SBH Abstract teaches "specific synthesis of oligonucleotides to beads."⁴ (Affymetrix Stmt. ¶ 19; cf. ¶ 12)

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⁴ Affymetrix's challenge that "the preferred system of 20 to 40 marker oligos" is not enabled is irrelevant. (Affymetrix Stmt. ¶ 19) The '432 patent claims do not require an encoding system of marker oligonucleotides.

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Illumina Resp.

Ex. V, Dower at 77:15-78:7; Illumina Resp. Ex. W, Crkvenjakov at 114:2-118:8; Illumina Resp. Ex. X, Drmanac at 96:5-10; Illumina Resp. Ex. Y, Beattie at 19:12-20:23) The prior art further confirms that it was well known how to attach oligonucleotides to beads *as early as 1974*. (See, e.g., Ex. Z) There is no genuine dispute about the fact that persons of ordinary skill in the art knew how to synthesize oligonucleotides on beads in 1989.

10. The declaration of Affymetrix's expert, Dr. Hubert Köster, is legally irrelevant and factually inconsistent with all of the evidence developed during discovery, and thus cannot create a genuine issue of material fact. *Invitrogen Corp. v. Clontech Laboratories, Inc.*, 429 F.3d 1052, 1080-81 (Fed. Cir. 2005) (finding expert declaration without any reasoning or supporting evidence merely speculative and insufficient to raise any genuine issue of material fact in light of all evidence to the contrary). His declaration can be quickly discounted based on the following facts and law:

- It is irrelevant that the SBH Abstract discloses no "proof of principle" working examples. See *Rasmusson*, 413 F.3d at

1326. Indeed, the '432 patent similarly does not provide any working examples (*see* ¶ 6 above);

- There is no need to put "multiple different oligonucleotide sequences on the same beads" since that is only one example of an encoding system, and Dr. Köster does not address the other examples (*see* ¶¶ 2, 8 above); and
- There is no requirement in the claims to "fix beads so as to form a monolayer in a manner that would allow functional decoding of the encoding system." (*see* ¶ 8 above)

11. Affymetrix, in fact, fails to point to any relevant evidence that disputes that the SBH Abstract disclosure enables one of ordinary skill in the art to practice *the asserted claims of the '432 patent*. Affymetrix's desperate digressions in arguing about Drs. Crkvenjakov and Drmanac's intent in disclosing their invention at scientific conferences (Affymetrix Stmt. ¶ 24), or whether these scientists were "crazy Yugoslavians" (Affymetrix Stmt. fn. 2), prove nothing except that it can create no genuine issue of material fact to preclude summary judgment.

III. The SBH Abstract is a Printed Publication under Federal Circuit Law

12. Five separate witnesses, one of whom is on Affymetrix's Scientific Advisory Board, all independently confirmed that the SBH Abstract was publicly disseminated at the Wolf Trap and Santa Fe conferences to about 80 and 165 attendees, respectively, in the fall of 1989, more than one year prior to the filing date of the '432 patent. (Illumina Br. at 4, 9 (Mathies, Drmanac, Crkvenjakov, Stodolsky, Beattie)) Without any dispute as to these facts, Affymetrix argues that more is required to prove that the SBH Abstract is a printed publication. (Affymetrix Stmt. ¶ 25-26) The Federal Circuit, however, has held that a printed reference actually distributed at a scientific conference is sufficient to be considered "publicly accessible" and prior

art under § 102(b). *M. I. T. v. AB Fortia*, 774 F.2d 1104, 1109 (Fed. Cir. 1985).⁵ Therefore, no genuine issues of material fact exist as to whether the SBH Abstract is a printed publication.

13. Affymetrix's arguments regarding the poster presentation (as opposed to the abstract) is yet another completely irrelevant argument. (Affymetrix Stmt. ¶ 27) The question on this motion is whether the SBH Abstract — not the poster or Dr. Crkvenjakov's replication of that poster — is a printed publication. It clearly is. Affymetrix's red herring argument does not create a genuine issue of material fact.


CONCLUSION

For all the reasons set forth herein and those set forth in Illumina's Opening Brief, Affymetrix fails to raise any genuine issues of material fact, and Illumina respectfully requests that this Court grant its motion for summary judgment of invalidity of all asserted claims of the '432 patent.

Dated: August 4, 2006

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⁵ Indeed, this jurisdiction has recognized that public accessibility can be demonstrated by either (1) actual distribution of the reference (such as "making the paper available at a conference where members of the interested public were told of the paper's existence and informed of its contents") or (2) cataloguing and shelving of the reference. *Ajinomoto Co., Inc. v. Archer-Daniels-Midland Co.*, 1998 WL 151411 at *37 (D. Del. 1998).

EXHIBIT T

**EXHIBIT REDACTED
IN ITS ENTIRETY**

EXHIBIT U

**EXHIBIT REDACTED
IN ITS ENTIRETY**

EXHIBIT V

UNITED STATES DISTRICT COURT
NORTHERN DISTRICT OF CALIFORNIA

--oOo--

AFFYMETRIX, INC., A DELAWARE)
CORPORATION,)

PLAINTIFF/COUNTER-DEFENDANT,)

vs.)

Case No. 04-901-JJF

ILLUMINA, INC., A DELAWARE)
CORPORATION,)

DEFENDANT/COUNTER-PLAINTIFF.)

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VIDEOTAPED DEPOSITION OF
WILLIAM J. DOWER, Ph.D

Wednesday, January 18, 2006
(Pages 1 - 278)

REPORTED BY: KATHLEEN WILKINS, RPR, CRR, CSR 10068
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WILLIAM J. DOWER, Ph.D

January 18, 2006

10:51:40 1 about polymer synthesis that dated back decades.

10:51:46 2 MS. TANG: Q. Okay. What was the
10:51:46 3 difference between what was known before and what is
10:51:50 4 shown in this patent?

10:51:52 5 MR. REED: Objection to form, calls for
10:51:53 6 speculation, lacks foundation, seeks a legal
10:51:55 7 conclusion, and calls for expert testimony.

10:51:59 8 THE WITNESS: And I would -- I think to
10:52:00 9 answer that fully, I would have to look more
10:52:03 10 carefully at what's in the patent to make that
10:52:07 11 comment.

10:52:07 12 MS. TANG: Q. Okay. Well, maybe -- maybe
10:52:09 13 we can approach this question by question.

10:52:12 14 A. Okay.

10:52:14 15 Q. Prior to VLSIPS, were you aware of whether
10:52:20 16 it was known how to make a surface with immobilized
10:52:25 17 oligos attached to that surface?

10:52:27 18 A. Yes.

10:52:28 19 MR. REED: Excuse me. Objection to form,
10:52:30 20 calls for speculation, seeks a legal conclusion,
10:52:32 21 lacks foundation, calls for expert testimony...

10:52:38 22 MS. TANG: Q. Okay. So also prior to
10:52:38 23 VLSIPS, was it known whether DNA oligos could be
10:52:41 24 fixed to a surface?

10:52:42 25 MR. REED: Same objections.

WILLIAM J. DOWER, Ph.D

January 18, 2006

10:52:43 1 THE WITNESS: Yes.

10:52:45 2 MS. TANG: Q. Okay. And was it known
10:52:48 3 prior to -- and maybe rather than saying VLSIPS, is
10:52:53 4 it fair to say 1990?

10:52:58 5 MR. REED: Objection to form.

10:52:59 6 THE WITNESS: VLSIPS was invented prior to
10:53:02 7 1990.

10:53:03 8 MS. TANG: Q. Okay. What about VLSIPS
10:53:05 9 with respect to DNA oligos?

10:53:08 10 MR. REED: Objection to form, lacks
10:53:09 11 foundation, seeks a legal conclusion, calls for
10:53:12 12 expert testimony.

10:53:13 13 THE WITNESS: Yes. I don't remember when
10:53:13 14 the conception of the oligos, actually.

10:53:17 15 MS. TANG: Q. Okay. So maybe -- let me
10:53:18 16 go back and ask these questions again.

10:53:21 17 In 1990, was it known how to immobilize
10:53:27 18 oligos on a surface not using VLSIPS?

10:53:31 19 MR. REED: Objection to form, lacks
10:53:32 20 foundation, calls for speculation, vague, seeks a
10:53:35 21 legal conclusion, calls for expert testimony.

10:53:38 22 THE WITNESS: There were methods that were
10:53:42 23 widely used for doing that.

10:53:44 24 MS. TANG: Q. Okay. In 1990 or before,
10:53:50 25 was it known how to fix DNA oligos on a surface --

CERTIFICATE OF REPORTER

I, Kathleen A. Wilkins, a Certified Shorthand Reporter, hereby certify that the witness in the foregoing deposition was by me duly sworn to tell the truth, the whole truth and nothing but the truth in the within-entitled cause;

That said deposition was taken down in shorthand by me, a disinterested person, at the time and place therein stated, and that the testimony of said witness was thereafter reduced to typewriting, by computer, under my direction and supervision;

That before completion of the deposition, review of the transcript ☒ was ☐ was not requested. If requested, any changes made by the deponent (and provided to the reporter) during the period allowed are appended hereto.

I further certify that I am not of counsel or attorney for either or any of the parties to the said deposition, nor in any way interested in the event of this cause, and that I am not related to any of the parties thereto.

DATED: 1/31/04

Kathleen Wilkins

Kathleen A. Wilkins, CSR No. 10068

EXHIBIT W

**EXHIBIT REDACTED
IN ITS ENTIRETY**

EXHIBIT X

**EXHIBIT REDACTED
IN ITS ENTIRETY**

EXHIBIT Y

IN THE UNITED STATES DISTRICT COURT
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v.

ILLUMINA, INC., a Delaware
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Defendant.

No. C4-901 JJF

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* * * * *

THE VIDEO DEPOSITION OF KENNETH L. BEATTIE, Ph.D.,

February 1, 2006

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KENNETH L. BEATTIE, Ph.D.

February 1, 2006

1 THE WITNESS: Right.

2 BY MS. TANG:

3 Q. And your approach used synthesis of
4 oligos on CPG beads, correct?

5 A. Right.

6 Q. And essentially your method was a
7 cheaper and faster combinatorial synthesis method?

8 MR. FAHNESTOCK: Objection to form.

9 THE WITNESS: Yes, I believe that,
10 yes.

11 BY MS. TANG:

12 Q. And would you agree that in this
13 late 1980's time frame it was commonly known how to
14 attach oligos to beads?

15 MR. FAHNESTOCK: Objection to form.

16 THE WITNESS: There were what was
17 commonly known as how to synthesize them on the beads
18 which they could be -- remain attached if you were
19 careful not to treat with the alkali at the end long
20 enough it would remove the protecting groups and
21 allow base pairing to occur, but if you did it longer
22 in a higher temperature then you would release them
23 from the beads.

24 But there were reagents available
25 commercially, notably from Cruachem sold them and

KENNETH L. BEATTIE, Ph.D.

February 1, 2006

1 also Glenn Research we bought a lot of reagents from
2 for derivatizing the ends of the oligonucleotides.
3 And you could put on it a functional group, a
4 carboxyl group or an amine group. Amine was the most
5 popular. And you could make them with the amine
6 group on either the three prime or five prime end.
7 And this allowed you to do, you know, attach these to
8 be to another support that had another reactive group
9 that would covalently attach to the amine. So that
10 was known at that time.

11 BY MS. TANG:

12 Q. Okay. So let me make sure I got
13 this right. In the late 1980's, it was known how to
14 attach an oligo to a solid support such as a bead
15 using a linkage such as an amide linkage?

16 A. That's true.

17 MR. FAHNESTOCK: Objection to form.

18 BY MS. TANG:

19 Q. And was it also known how to attach
20 an oligo to a bead using a streptavidin biotin
21 attached?

22 A. I think so because that was a very
23 commonly used reaction for other purposes.

24 MS. TANG: Now, I'd like to turn to
25 a document.

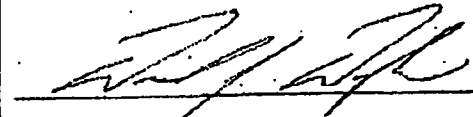
C E R T I F I C A T E

STATE OF TENNESSEE:

COUNTY OF KNOX:

I, David J. Doyle, Court Reporter and Notary Public, do hereby certify that I reported in machine shorthand the foregoing proceedings; that the foregoing pages, numbered 1 to 158, inclusive, were typed by me using computer-aided transcription and constitute a true and accurate record of said proceedings.

I further certify that I am not an attorney or counsel of any attorney or counsel connected with the action, nor financially interested in the action. Witness my hand and official seal this 5th of February, 2006.



David J. Doyle

Court Reporter

And Notary Public at Large.

My Commission Expires: 06/09/09

EXHIBIT Z

United States Patent [19][11] **4,046,750****Rembaum** **BEST AVAILABLE COPY**[45] **Sept. 6, 1977**[54] **IONENE MODIFIED SMALL POLYMERIC BEADS**[75] **Inventor:** Alan Rembaum, Altadena, Calif.[73] **Assignee:** California Institute of Technology, Pasadena, Calif.[21] **Appl. No.:** 510,786[22] **Filed:** Sept. 30, 1974[51] **Int. Cl.:** C08F 8/30[52] **U.S. Cl.:** 526/310; 204/159.22; 210/37 R; 424/2; 424/25; 424/78; 424/271; 424/329; 260/33.2 R; 260/42.21; 260/80.3 N; 260/86.1 R; 260/86.1 N; 260/86.1 E; 526/292; 526/296; 526/328[58] **Field of Search** 260/80.73, 80.3 N, 86.1 R, 260/86.1 N, 86.1 E[56] **References Cited****U.S. PATENT DOCUMENTS**

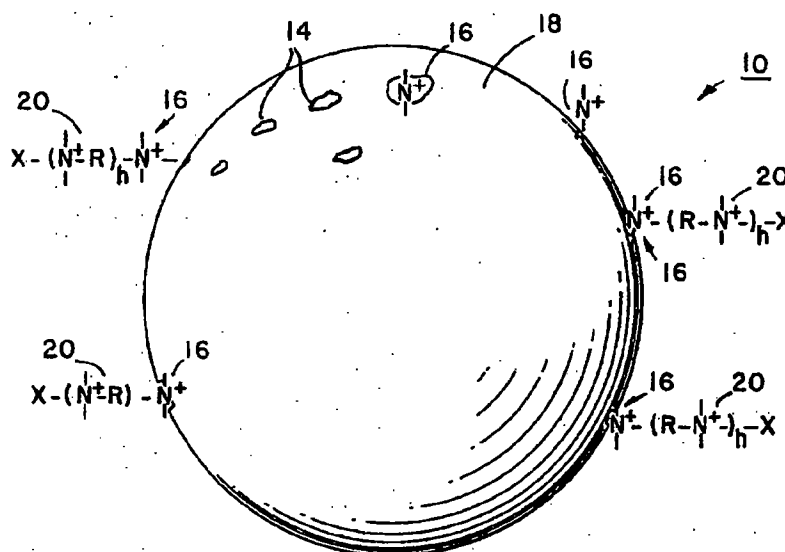
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Primary Examiner—John Kight, III
Attorney, Agent, or Firm—Marvin E. Jacobs

[57] **ABSTRACT**

Linear ionene polyquaternary cationic polymeric segments are bonded by means of the Menshutkin reaction (quaternization) to biocompatible, extremely small, porous particles containing halide or tertiary amine sites which are centers for attachment of the segments. The modified beads in the form of emulsions or suspensions offer a large, positively-charged surface area capable of irreversibly binding polyanions such as heparin, DNA, RNA or bile acids to remove them from solution or of reversibly binding monoanions such as penicillin, pesticides, sex attractants and the like for slow release from the suspension.

8 Claims, 2 Drawing Figures

U.S. Patent

Sept. 6, 1977

4,046,750

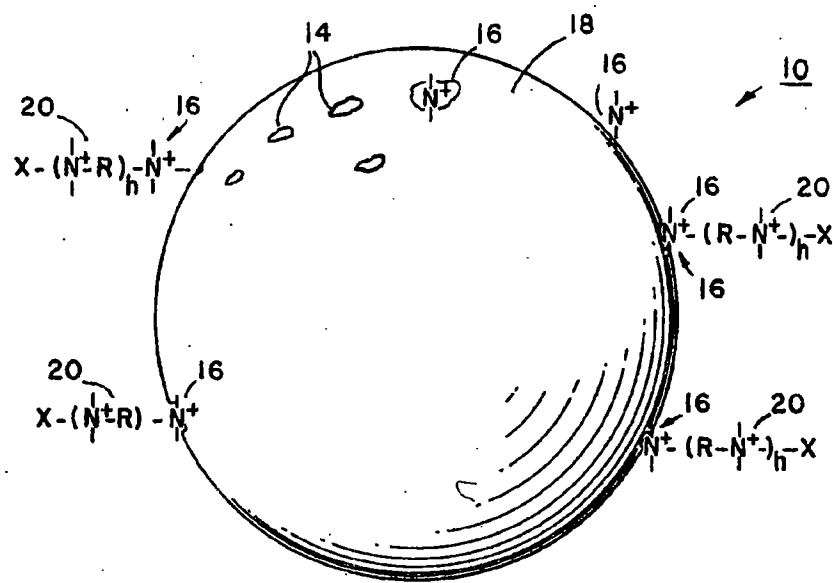


FIG. 1

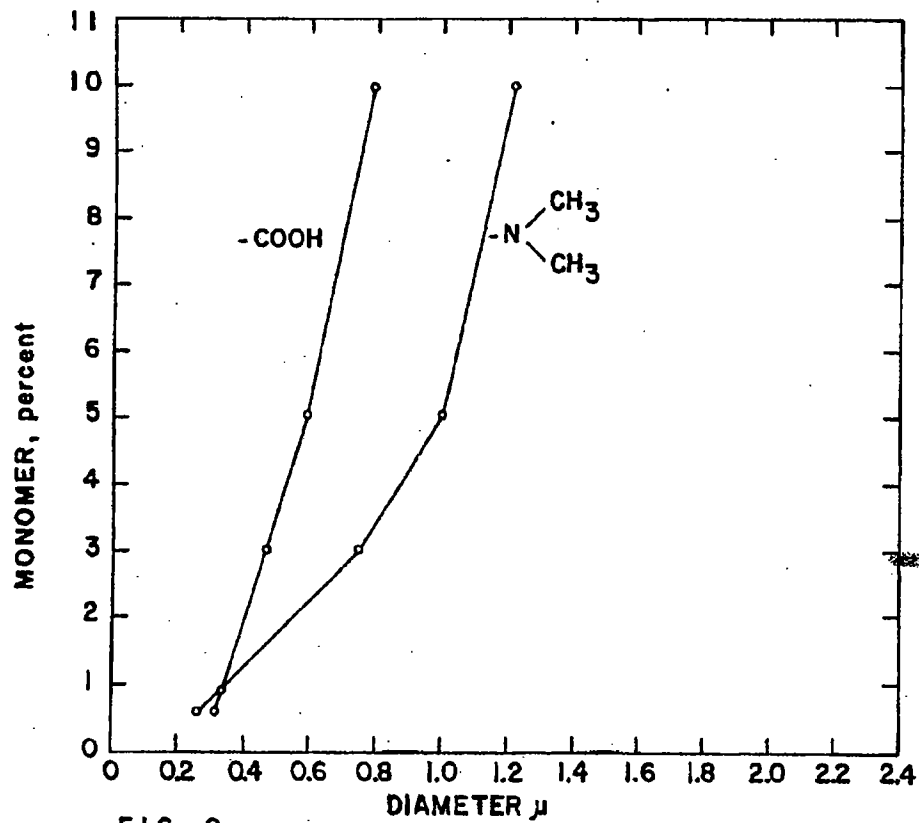


FIG. 2

1

4,046,750

2

IONENE MODIFIED SMALL POLYMERIC BEADS

ORIGIN OF THE INVENTION

The invention described herein was made in the performance of work under a NASA contract and is subject to the provisions of Section 305 of the National Aeronautics and Space Act of 1958, Public Law 83-568 (72 Stat. 435; 42 USC 2457).

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to small, polymeric beads and, more particularly, to linear ionene modified beads for use in binding small and large anionic compounds.

2. Description of the Prior Art

It is believed that certain clinical hemorrhagic states are associated with a heparin-like substance in the blood. In addition, the value of antiheparin agents lies also in the treatment of post-partum hemorrhage and the restoration of normal blood coagulability after open heart surgery and after hemodialysis, where administration of relatively large doses of heparin is a common practice. Protamine sulfate and toluidine blue, neither of which are free of toxic effects, are clinically used as antiheparin agents.

Polycations obtained by the Menshutkin reaction of aliphatic diamines with aliphatic dihalides or by homopolymerization or dimethylamino-n-alkyl halides are referred to as ionenes. The ionenes constitute a unique system because of their structure, their distances between positive charges, their counterions and their molecular weight can be varied systematically. They form strong, insoluble complexes with heparin, the concentration of which can be determined by a simple potentiometric titration.

Studies have shown that the heparin concentration in water in low ionic strength can be determined by following the pH changes of a heparin solution when ionenes are gradually added to it. Quantitative yields of heparin ionene complexes are obtained at the neutralization point. The amount of ionene necessary to neutralize a given amount of heparin depends on the charge density of the ionene and can be determined by means of this pH titration. In addition, this procedure also offers information of the stoichiometry of polycation polyanion complexes and on the charge density of polyelectrolytes in general.

Although most ionene structures have antiheparin activity, extensive investigations of toxicology and effects on the circulatory system in laboratory animals were carried out only with 6,3-ionene bromide referred to as "Polybrene." The latter was found to be more toxic (i.v. LD₅₀, 28 mg/kg in mice and 20 mg/kg in rats; the i.p. LD₅₀ in mice is 61.5 mg/kg) than toluidine blue (i.v. LD₅₀, 45 mg/kg) and protamine sulfate (i.v. LD₅₀, 44 mg/kg). However, cumulative i.v. doses of 6,3-ionene bromide up to 5 mg/kg as 1 percent solutions could be given rapidly to anesthetize dogs without markedly affecting either the respiration or circulation, i.e., without toxic symptoms.

Heparin offers a protective action in neutralizing the toxicity of 6,3-ionene bromide in both mice and dogs. Thus pre-treatment of mice with heparin enabled them to survive doses of three times the LD₅₀ values with only mild toxicity symptoms.

SUMMARY OF THE INVENTION

It has now been discovered in accordance with the invention that ionenes covalently bound to small, polymeric spheres can be utilized for the efficient removal of heparin from aqueous solution or for general use in binding anions and polyanions of diverse nature in separation, analytical, diagnostic and clinical applications. The cationic modified beads provide a large surface area and form water-insoluble complexes with anions, removing them from solution.

The uniformly-shaped, porous beads are formed by the aqueous suspension copolymerization of a halo- or dimethylaminosubstituted acrylic monomer and a cross-linking agent. Cross-linking proceeds at high temperature above about 50° C or at lower temperature with irradiation. Beads of even shape and size of less than 2 micron diameter are formed in the presence of an aqueous soluble polymer such as a polyether and of a larger size in the absence of a polyether or in the presence of a soluble monomer such as 2-aminoethylmethacrylate or allyl amine. The beads are separated and reacted with a mixture of a ditertiary amine and a dihalide or with a dimethylaminoalkyl halide to attach ionene segments to the halo or tertiary amine centers on the beads. The insoluble cationic modified beads are readily separated from soluble ionene homopolymer that is formed.

Large sized beads of the order of 50 micron diameter will find use in affinity or pellicular chromatography. The column of beads preferentially removes heparin from its mixture with polycations or neutral substances such as proteins or serums. The cationic beads can be used in the separation of cholesterol precursors such as bile acid from bile micellar suspensions.

The beads bind RNA or DNA irreversibly and smaller size beads have been found to penetrate the membrane of living cells and enter the nucleus thereof. The cationic modified beads also show cytotoxic activity toward malignant cells.

The beads form weak reversible complexes with anionic compounds containing 1-5 anionic groups, permitting slow release of the anionic compound from suspension. Thus, suspensions will find use in the sustained release of nutrients, hormones, vitamins, pharmaceuticals, sex attractants, pesticides in clinical agriculture and maricultural applications. Penicillin is found to be slowly released from a suspension of ionene treated beads. The binding of cytotoxic drugs such as Methotrexate to the beads should provide increased activity due to the cytotoxic activity of the ionene.

The cationically charged spheres can also be used as markers of negative sites on living cells or tissue and the charged spheres undergo phagocytotic action by these cells. The presence of OH, COOH and amine groups on the beads permits covalent binding of biomolecules such as haptens, enzymes, antibodies or lectins to the beads by means of cyanogen bromide, carbodiimide or glutaraldehyde reactions.

The labeled beads can be utilized for the diagnosis of conditions such as hepatitis, gonorrhea, rheumatoid arthritis, streptococcus infections and pregnancy by mixing the labeled beads with a body serum and observing whether the beads bind to specific antigen sites causing precipitation or agglutination. The labeled beads may also be utilized in the treatment of the diseased condition by the use of the bound specific biomolecular agent to direct the bead to the desired cell

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and the toxic action of the polyquaternary function on the cell.

The toxicity of the ionene function is considerably decreased if not eliminated by complexation with the above-mentioned polyanions. The complex is taken up by living cells easier than the separate components since in the bead complex all charges are neutralized. This activity is of particular interest since it has previously been discovered that ionene-RNA complexes have antiviral activity.

These and many other attendant advantages will become apparent as the invention becomes better understood by reference to the following detailed description when considered in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic view of an ionene-modified bead according to the invention; and

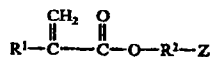
FIG. 2 is a graph illustrating the effect of acrylic acid or dimethylaminoethylmethacrylate monomer concentration on particle size.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The bead 10 as shown in FIG. 1 is a small, essentially spherical particle having pores 14 and containing ionene reactive halide or tertiary amine groups 16 throughout the bead 10 and on the surface 18. Ionene segments 20 extend from the surface of the bead.

The beads are prepared by the aqueous suspension polymerization of a monounsaturated, bromo, chloro, iodo or tertiary amine substituted acrylic monomer and 0.1 to 30% by weight of a cross-linking agent. Polymerization proceeds at a temperature above 50° C, preferably 70° C, to reflux in the presence or absence of a free radical catalyst or at a lower temperature of -70° C to 70° C with application of high energy radiation to the polymerizable mixture. Smaller beads of uniform spherical shape of the order of 0.1 to 2 microns or smaller are favored in the presence of 0.1 to 5% by weight of a water soluble polymeric suspending agent such as polyether having a molecular weight from 300,000 to 6,000,000 such as polymers of ethylene oxide, propylene oxide or mixtures thereof.

The ionene reactive monomer is suitably a compound of the formula:



where R¹ is hydrogen or lower alkyl of 1-8 carbon atoms, R² is alkylene of 1-12 carbon atoms and Z is chloro, bromo, iodo or



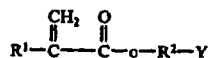
where R³ and R⁴ are alkyl of 1-3 carbon atoms. Suitable compounds are dimethylaminoethylmethacrylate and 2-bromoethylmethacrylate.

The ionene reactive monomer may be mixed with up to 97% by weight of a compatible comonomer such as a lower alkyl methacrylate, acrylic acid, methacrylic acid, styrene, vinyl toluene, acrylamide or hydroxyl

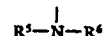
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alkyl or amino alkyl substituted acrylates of the formula:



where R¹ and R² are as defined above and Y is OH or



where R³ is hydrogen and R⁴ is hydrogen, lower alkyl or lower alkoxy of 1-8 carbons atoms. Representative compounds are hydroxyethyl-methacrylate, hydroxypropylmethacrylate, 2-aminoethyl methacrylate.

The cross-linking agent is present in the polymerizable mixture in an amount from 0.1 to 30%, preferably 1-6% by weight, and is a polyunsaturated compound such as a diene or a triene capable of addition polymerization with the unsaturated group of the monomer. Suitable compounds are low molecular weight polyvinyl compounds such as ethylene glycol dimethacrylate, divinyl benzene, trimethylol propane trimethacrylate and N,N'-methylene-bis-acrylamide (BAM).

A commercial form (94%) of hydroxyethylmethacrylate (HEMA) and hydroxypropyl methacrylate (HPMA) as supplied, contains small amount of methacrylic acid, hydroxyalkoxyalkylmethacrylate and dimethacrylates - ethylene glycol dimethacrylate in HEMA and propylene glycol dimethacrylate in HPMA. HPMA is generally a mixture in which the principal monomers comprise 68-75% of 2-hydroxypropyl and 25-32% of 1-methyl-2-hydroxyethylmethacrylate. Typical compositions in weight percentage follows:

Compound	HEMA 94%	HPMA 94%
Hydroxyalkylmethacrylate	86	87
Higher boiling methacrylate, principally hydroxyalkoxy- alkylmethacrylate	6	5
Methacrylic Acid	3.5	4.5
Dimethacrylate	1.5	0.7

The monomers are diluted in aqueous medium at a level of from 0.5 to 50% by weight, preferably 1-20% by weight. The aqueous medium comprises water and the soluble polymer. Water soluble polymeric stability agents such as polyvinyl, pyrrolidone or polyether may be present in an amount as low as 0.05 weight percent. Amounts above 5% are believed unnecessary and require added time and effort to remove the polymer from the final beads.

Finely and uniformly shaped and sized beads have consistently been produced in an aqueous medium containing a stabilizing agent such as a polyether. The polyethers generally have a molecular weight from 300,000 to 5,000,000, preferably 400,000 to 2,000,000, and are polymers of alkylene oxides such as ethylene oxide, propylene oxide or their mixtures. Polyethylene oxides are preferred due to their solubility in water.

The polymerization proceeds with or without catalyst and with or without stirring with application of heat to the mixture at a temperature of from 70° C to reflux, generally about 100° C or with application of high energy radiation capable of generating free radi-

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cals and initiating polymerization and forming cross-linking bonds between olefinic groups. Surprisingly mono-dispersed beads of fairly even size range are formed without the presence of an emulsifying agent. The presence of the dimethylamino monomer stabilized the beads in that they do not coalesce in suspension as compared to beads containing carboxyl groups. Polymerization proceeds by application of 0.05 to 1.0 megarads of radiation from a cobalt gamma source at a temperature of 0° C to 70° C. The reaction is preferably conducted under oxygen excluding conditions, generally by applying vacuum to the reaction vessel or by bubbling inert gas such as nitrogen through the mixture. A free radical catalyst such as ammonium persulfate and additional agents such as sodium lauryl sulfate may be present in the polymerizable mixture.

After polymerization has proceeded to completion, the polymerization mixture is diluted with hot water and filtered and washed with boiling water to remove the polyether or simply centrifuged without dilution. The dry material in over 90% yield is in the form of separate round beads or agglomerates of beads. Agglomerates, if present, are subdivided into beads mechanically by dispersion in a non-solvent liquid, crushing or grinding. The beads are uniformly sized and at least 80 percent, and preferably at least 90 percent, of the beads are of a uniform diameter less than 5 microns, preferably from 500A. to 2 microns. The cross-linked porous beads are insoluble and swellable in water and are insoluble in common inorganic and organic solvents.

Specific examples of practice follow.

EXAMPLE 1

The following aqueous mixture was prepared.

Component	Weight, gm
HEMA (Freshly distilled containing 1.5% ethylene dimethacrylate)	40
Trimethylol propane trimethacrylate (TPT)	6.0
Polyethylene oxide (M.W. 10 ⁶)	4.0
Dimethylaminoethyl methacrylate	10
Water to one liter	

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The mixture was nitrogen inerted and 0.1 megarads of radiation was applied to the mixture at room temperature from a cobalt gamma source. The beads were filtered, washed with boiling water several times and centrifuged to provide a 99 percent yield. Under scanning electron microscope, the diameter of over 90 percent of the beads was determined to be from 1-2 microns. The copolymer beads contain hydroxyl as well as dimethylamino groups. The procedure was repeated at 0° C in ice bath with 0.2 and 0.4 megarads with similar results.

EXAMPLE 2

The following aqueous mixture was prepared.

Component	Weight, gm
HEMA	60
Dimethylaminoethyl methacrylate	10
TPT	2.0
Polyethylene oxide (M.W. 10 ⁶)	4.0
Water to one liter	

The mixture was nitrogen inerted and subjected to 0.4 megarads of cobalt gamma radiation. Individual beads of about 1 micron diameter were produced.

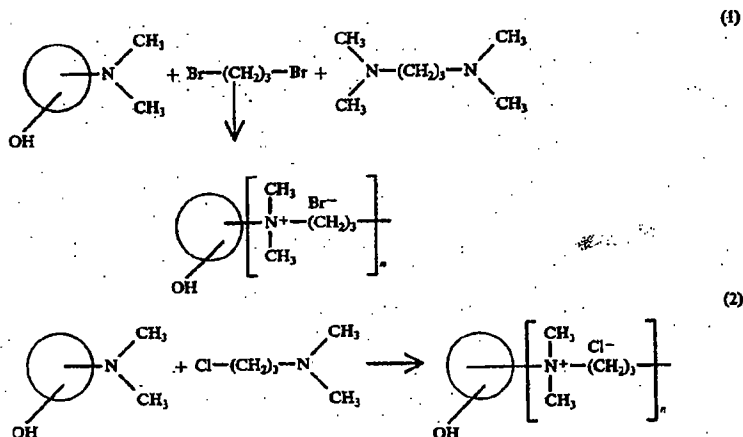
EXAMPLE 3

The following aqueous mixture was prepared.

Component	Weight, gm
HEMA	35
Dimethylaminoethyl methacrylate	15
N,N'-methylene bis acrylamide	6.0
Polyethylene oxide (M.W. 10 ⁶)	4.0
Water to one liter	

The mixture was polymerized under the condition of Example 2 to yield individual beads having a well characterized diameter of about 0.9 microns.

The tertiary amine or halogen modified beads are then reacted with linear ionene forming reactants such as (1) α,ω -dihalo alkane or (2) with dimethylamino- η -alkyl chloride. The two reactions for a 3-Ionene Bromide can be represented as follows:



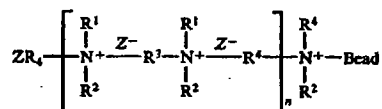
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Both reactions yielded aqueous suspensions of spheres (with 3-ionene on the surface) observable under an ordinary light microscope. The presence of ionenes can be demonstrated qualitatively by the use of anionic dyes; Trypan blue and Eosin Y are removed from aqueous solutions by means of ionene spheres.

The ionene polymers of interest in this invention are water-soluble, linear polymers, without cross-linking or branching. The polymer segments have a molecular weight from 500 to 100,000, generally from 1,500 to 60,000, and have an average charge of at least one intra polymeric quaternary nitrogen for an average of every twelve chain atoms.

The ionene modified beads have the general structure:



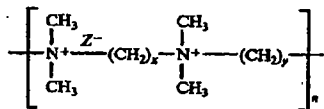
where R¹ and R² are methyl, R³ and R⁴ are divalent aliphatic, aromatic or heterocyclic groups containing at least 3 carbon atoms, or R³ combined with R¹ and R² forms a cyclic group and Z⁻ is an anion, generally chloro, bromo or iodo.

Aliphatic ionene polymers in which R³ and R⁴ are the same polymethylene group of the formula (CH₂)_x where x is 3 or more than 6 can be prepared by homopolymerization of tertiary amino alkyl halides of the formula



in accordance with the procedure disclosed in copending application Ser. No. 280,649, filed Aug. 14, 1972. Values of x between 4 and 7 result in cyclic products. Generally the polymerization is conducted in water at a concentration of monomer above 3 molar, at a temperature from 80–110° C under oxygen excluding conditions.

Ionenes can also be prepared by the copolymerization of ditertiary amines and dihalo organic compounds. This reaction permits the synthesis of a variety of linear polymers in which the distance between positive nitrogen centers can be varied at will. With aliphatic ionenes of the formula:



the values of x and y between 3 and 16 must also be selected to avoid formation of cyclic compounds, as disclosed by Rembaum et al., *Macromolecules* 5 261 (1972), the disclosure of which is incorporated herein by reference.

Well defined conditions of synthesis relating to formation of relatively high molecular weight ionenes are disclosed by Rembaum et al., *J. Polym. Sci., Part B* 6 (1968), the disclosure of which is incorporated herein by reference. Generally, high molecular weight ionene polymers are prepared in a 0.1 to 2.5 molar solution of a ditertiary amine and a dihalo organic compound in solvent at temperatures below about 50° C. Higher polymerization rates occur in polar organic solvents such as dimethyl formamide (DMF), dimethyl sulfoxide

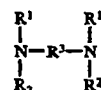
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(DMSO), methanol, preferably a mixture of DMF and methanol.

The dihalo organic material is a compound of the formula ZR⁴Z where Z is chloro, bromo or iodo, where R⁴ is a divalent organic radical such as alkylene, arylene, alkarylene or aralkylene. Hydrocarbon R⁴ groups may also be interrupted with atoms such as nitrogen, oxygen or sulfur and may be substituted with diverse pendant groups that do not interfere with the polymerization reaction or activity of the polymer or promote undesirable side effects during use.

Representative dihalo organic compounds are α,ω-chloro or bromo terminated compounds such as 1,3-dichloropropane, 1,3-dibromopropane, 1,4-dichlorobutane, 1,4-dibromobutane, 1,4-dichloro-2-butene, 1,4-dibromo-2-butene, 1,4-dibromo-2,3-dihydroxy butane, 1,5-dichloropentane, 1,6-dibromohexane, 1,8-dichlorohexane, 1,10-dichlorodecane, and 1,16-dichlorohexadecane. The alkenylene compounds are more reactive than the corresponding saturated compounds. Dihal aromatic compounds such as o, m, and p-dichloro or -dibromo xylene may also be utilized.

The diamine reactant for the copolymerization reaction may be represented by the formula:



where R³ is aliphatic, aromatic, heterocyclic or R³ when combined with R¹ and R² forms a cyclic group. Representative compounds are N,N,N',N'-tetramethyl-1,3-diamino propane, N,N,N',N'-tetra-methyl-1,3-hexamethylene diamine (THD) and N,N,N',N'-tetramethyl-1,10-decamethylene diamine. Examples of heterocyclic or aromatic compounds are 1,2-bis-(4-pyridyl)-ethane, -propane or -butane, dipyridyl, diazo-bicyclooctane or tetramethyl diamino, diphenyl methane.

EXAMPLE 4

Spherical particles (1.66 microns in diameter) containing 7.64×10^8 dimethylamino groups per sphere were stirred with 1,3-dibromopropane and 1,3-tetramethylamino propane at room temperature in dimethylformamide-methanol mixture (1:1 by volume) for 24 hours. The reaction product after addition of water was centrifuged. The centrifugation in presence of water was repeated until the supernatant was free of bromide ions. The spheres contained 2.9 meq of bromine per gram.

EXAMPLE 5

The spherical dimethylamino-substituted particles of Example 4 were suspended in an aqueous medium containing dimethylaminopropylchloride in 5 molar concentration. The suspension was heated at 90° C to 100° C while bubbling nitrogen through the suspension to exclude oxygen. The beads recovered as in Example 4 contained 2.1 meq of chlorine per gram of beads.

EXAMPLE 6

An aqueous suspension of the polycation spheres of Examples 4 and 5 (1 cc, 28 mg/cc; 7×10^9 spheres/cc) was added to 20 ml of a heparin solution (1 mg/ml). After stirring the mixture for 15 minutes and filtration, the filtrate contained 0.04 mg of heparin per cc. By repeating this experiment under identical conditions but

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using 2 cc of suspended, charged spheres, no heparin could be detected in the filtrate by means of Azure A. The presence of positive charges on the spherical particles was ascertained by reaction with Eosin Y. The latter is an acidic dye which combines with 3,3-ionene to form an insoluble red precipitate. The ionene spheres were stirred in an aqueous solution with Eosin Y for 10 minutes and then centrifuged in distilled water ten times. The spheres remained dark red. Polyhydroxyethylmethacrylate spheres without dimethylamino groups served as control. After reaction with Eosin y and centrifugation, they were free of dye and appeared white.

The percentage of halide is a measure of the length of the ionene segments attached to the beads. Since the beads are porous, some ionene reaction with interior halogen or dimethylamino sites can be expected. However, the majority of the reaction is expected to proceed by linear addition to the surface sites. The amount of ionene is also dependent on the amount of amine or halogen functionality present in the bead and the size of the bead. For beads in the 0.5 to 1.5 micron range polymerized with 4-20% tertiary amino alkyl acrylate, a typical percentage for bromine is from about 1 meq to 30 meq per gram of beads.

The amount of heparin complexed with the ionene spheres in Example 6 is shown in the following table.

Table 1

Removal of Heparin from Aqueous Solutions		
	Halide meq/g	mg Heparin complexed/g of spheres
Ionene spheres (Ex. 4)	2.9	335.7
Ionene spheres (Ex. 5)	2.1	300.5

EXAMPLE 7

Preparation of microspheres containing dimethylamino functional groups.

Freshly distilled, specially purified (about 99 percent pure) 2-hydroxyethyl methacrylate (a) containing 0.37 ethylene glycol dimethacrylate and less than 0.01 of methacrylic acid and freshly distilled 2-dimethylaminoethyl methacrylate or methacrylic acid (b) were mixed in the proportion of 4 of (a) to 1 of (b) by weight. The mixture containing 2% weight of BAM and 0.4% w/v of high molecular weight polyethylene oxide was used to make up 0.5, 3, 5 and 10% w/v solutions in distilled water.

The solutions were subjected to a Co α irradiation dose of 0.2 megarads. The irradiated samples were centrifuged five times at 5 to 10,000 rpm for 15 minutes. The supernatant liquor was discarded after each centrifugation and the solid was redispersed in distilled water. The diameter of the microspheres was determined by scanning electron microscopy. In FIG. 2 are shown the sizes of the microspheres as a function of total monomer concentration. The dimethylamino content varied from 1.71 to 2.2 weight % compared to a value of 1.77 weight % theoretical content.

By eliminating the PEO from the reaction mixture, the size of the microspheres could be considerably increased.

The size of the spheres obtained is somewhat larger in the bead containing dimethylamino end groups and would be of intermediate size in a copolymer containing both methacrylic acid and 2-dimethylaminoethyl methacrylate. Use of a water-insoluble monomer such as

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methyl methacrylate further decreases the size of the beads.

EXAMPLE 8

A water suspension containing 1g of microspheres of 1.2 μ in diameter prepared in Example 7 was centrifuged and resuspended in a mixture of 50 ml dimethylformamide and methanol (4:1 v/v), 1,3-dibromopropane (2.02g) and N,N,N',N'-tetramethyl propane diamine (1.3g) were added and the mixture was stirred until the contents solidified. After leaving standing for 48 hours, distilled water was added to the mixture which was centrifuged until the supernatant did not show a precipitate or cloudiness on addition of 1 M solution of ammonium perchlorate.

EXAMPLE 9

10 ml of a 1.2 micron ionene bead suspension (20 mg/ml) prepared in Example 8 were combined with 5 ml of ox bile extract. The beads absorbed oily material and separated the bile acid and possibly some cholesterol from the aqueous micellar solution and were filtered and removed. The bile acid content of the extract was considerably reduced. Since the ionenes are bound to colloidal-sized, insoluble particles, it is believed certain that they will go through the gastrointestinal tract without passage through tissue.

EXAMPLE 10

To test the activity of ionenes on bile acids, 50 mg portions of high molecular weight, non-toxic 3,3-Ionene Bromide and 6,10-Ionene Bromide were added to 5 cc of ox bile extract. An oily layer formed in each case containing the ionene complexed to the bile acids.

EXAMPLE 11

5 mg of a sodium salt of penicillin was added to a 50 mg/100cc aqueous suspension of 1.6 micron 3-ionene modified beads. The antibacterial activity of the water increased with time demonstrating the sustained slow release of penicillin from its covalent complex with the ionene bead.

EXAMPLE 12

Normal thymocyte cells of mouse origin were incubated with a suspension of 1.6 micron beads modified with 3-ionene containing 10⁵ beads/ml and 10⁴ cells/ml. After one hour, there was no evidence of cytotoxic activity.

EXAMPLE 13

A suspension of 3-ionene beads as in Example 12 was incubated with a 10⁴ cell/ml suspension of EL-4 leukemic cells of mouse origin. After 10 minutes, Trypan blue dye was added and by visual observation it was determined that all cells in contact with the beads were dead. The results were confirmed with a 3,3-Ionene Bromide polymer.

Since the concentration of ionene on the bead is strictly controlled, the beads can be added to mixtures of normal and diseased cells without danger of cytotoxic action on the normal cells. The small, insoluble bead particles are readily dyed and observed. The small beads, especially when the ionene functionality is complexed, may be well tolerated in blood or serum living animals.

It is to be understood that only preferred embodiments of the invention have been described and that

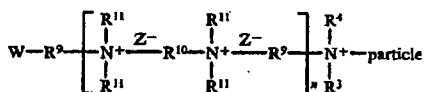
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numerous substitutions, alterations and modifications may be made without departing from the spirit and scope of the invention as defined in the following claims.

What is claimed is:

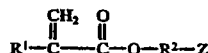
1. A composition of matter comprising: small, synthetic, organic, polymeric spherical particles having a diameter from 100 Å. to 100 microns which are the cross-linked, addition polymerization product of a mono-unsaturated, acrylic monomer substituted with ionene reactive chloro, bromo, iodo or tertiary amine sites with 0.1 to 30% by weight of a diene or triene capable of addition polymerization with the unsaturated group of the acrylic monomer and having covalently bonded to at least one of said sites linear, polyquaternary ionene segments having an average charge of at least one intrapolymeric quaternary nitrogen for an average of every twelve chain atoms such that the halogen content of the segments is from 1 meg to 30 meg per gram of particle to form a particle of the structure:



where R^{11} is methyl, R^9 and R^{10} are divalent aliphatic, aromatic or heterocyclic groups containing at least 3 carbon atoms, or R^9 combined with R^{11} form a cyclic group, W is chloro, bromo, or iodo and n is an integer.

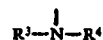
2. A composition according to claim 1 in which the particle has a diameter from 500 Å. to 2 microns.

3. A composition according to claim 1 in which the acrylic monomer is present in an amount of from 2-30% by weight of the particle and is selected from compounds of the formula:



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where R^1 is hydrogen or lower alkyl of 1-8 carbon atoms, R^2 is alkylene of 1-12 carbon atoms and Z is chloro, bromo, iodo or

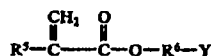


where R^3 and R^4 are alkyl of 1-3 carbon atoms.

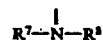
4. A composition according to claim 3 in which the acrylic monomer is selected from dimethylaminoethylmethacrylate and 2-bromoethylmethacrylate.

5. A composition according to claim 3 in which the particle contains up to 97% by weight of at least one compatible comonomer selected from lower alkyl methacrylate, acrylic acid, methacrylic acid, styrene, vinyl toluene, acrylamide, hydroxyalkyl acrylate, or amino alkyl acrylates.

6. A composition according to claim 5 in which the comonomer is selected from compounds of the formula:



where R^5 is hydrogen or lower alkyl of 1-8 carbon atoms, R^6 is alkylene of 1-12 carbon atoms and Y is OH or



where R^7 is hydrogen and R^8 is hydrogen, lower alkyl of 1-8 carbon atoms or lower alkoxy of 1-8 carbon atoms

7. A composition according to claim 6 in which the comonomer is selected from hydroxyethylmethacrylate, hydroxypropylmethacrylate and 2-aminoethylmethacrylate.

8. A composition according to claim 1 in which R^9 and R^{10} are alkylene from 3 to 16 carbon atoms and said segments have a molecular weight from 1,500 to 60,000.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 4,046,750

DATED : September 6, 1977

INVENTOR(S) : ALAN REMBAUM

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 2, line 14, change "dimethylaminosubstituted" to
-- dimethylamino-substituted".

Column 2, line 39, change "beadsalso" to -- beads also --.

Column 4, line 5, in the formula, change "o" to -- O --.

Column 4, line 50, between "the" and "soluble" insert -- water --.

Column 6, line 37, change "10hu 6" to -- 10^6 --.

Column 7, line 7, after "of interest" delete "in this interest".

Column 7, line 65, change "compoundin" to -- compound in --.

Column 8, line 30, in the formula, change " R_2 " to -- R^2 --.

Column 10, line 25, change "colldoial" to -- colloidal --.

Signed and Sealed this

Fourteenth Day of February 1978

[SEAL]

Attest:

RUTH C. MASON

Attesting Officer

LUTRELLE F. PARKER

Acting Commissioner of Patents and Trademarks

CERTIFICATE OF SERVICE

I hereby certify that on the 21st day of August, 2006, I caused to be electronically filed the foregoing document, **REDACTED VERSION OF ILLUMINA'S RESPONSE TO AFFYMETRIX'S STATEMENT OF DISPUTED MATERIAL FACTS REGARDING ILLUMINA'S MOTION FOR SUMMARY JUDGMENT OF INVALIDITY OF THE ASSERTED CLAIMS OF THE '432 PATENT**, with the Clerk of the Court using CM/ECF which will send notification of such filing to the following:

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